

CLAIMS

Sub C4

1. A DNA construct comprising:

- (1) a selective marker gene,
- (2) a galactose-inducible growth inhibition sequence,
- (3) a pair of FRT sequences in the same orientation flanking (1) and (2), and
- (4) a DNA fragment capable of recombining with a yeast chromosomal DNA located at each end of (3), wherein said FRT sequences contain the following sequence:

5'-GAAGTTCTATAC TTTCTAGA GAATAGGAACCTTC-3' (SEQ ID NO: 1)

inverted spacer inverted
repeat (1) sequence repeat (1)

or a sequence substantially identical to said sequence, provided that in each member of said pair of FRT sequences, the inverted repeat distal from the flanked selective marker gene and growth inhibition sequence has at least one but no more than six nucleotides deleted on the side distal from the spacer sequence.

2. The DNA construct of claim 1 wherein a gene of interest is inserted between the DNA fragment capable of recombining with a yeast chromosomal DNA and a FRT sequence adjacent to said fragment.

Sub C5

3. A method for transforming a yeast of the genus *Saccharomyces*, comprising:

- (1) transferring the DNA construct of claim 1 into yeast cells to integrate said DNA construct into a yeast chromosome by recombination between a DNA fragment capable of recombining with a yeast chromosomal DNA present in said

DNA construct and the yeast chromosomal DNA,

(2) selecting yeast cells transfected with said DNA construct based on the expression of a selective marker gene contained in said DNA construct,

(3) culturing said cells in a non-selective medium to induce recombination between a pair of FRT sequences contained in said DNA construct, thereby excising the selective marker gene, and

(4) culturing said cells in a medium containing galactose to select growable yeast cells.

4. The method of claim 3 comprising inserting a gene of interest into a yeast chromosome using a DNA construct containing said gene of interest inserted between a DNA fragment capable of recombining with a yeast chromosomal DNA and an FRT sequence adjacent to said fragment.

5. The method of claim 4 wherein the step of claim 4 is repeated to introduce a plurality of genes of interest.

6. A yeast of the genus *Saccharomyces* transformed by the method of any one of claims 3 to 5.

7. A method for producing a beer comprising using the yeast of the genus *Saccharomyces* of claim 6.

8. A beer obtained by the method of claim 7.

Added c7